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Phase-transition properties of glycerol–dipalmitate lipid bilayers investigated using molecular dynamics simulation

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ABSTRACT

The phase- and phase-transition properties of glycerol–dipalmitate (GDP) bilayer patches are investigated using molecular dynamics simulations. This permits to characterize the influence of introducing a second aliphatic lipid tail by comparison to previously reported simulations of glycerol-1-monopalmitate (GMP). To this purpose, a set of 67 simulations (up to 300 ns duration) of $2 \times 8 \times 8$ GDP bilayer patches are performed, considering the two GDP isomers glycerol-1,3-dipalmitate (13 GDP) and glycerol-1,2-dipalmitate (12 GDP; racemic), two hydration levels (12GDP only), and temperatures in the range 250–370 K. In agreement with experiment, the GDP simulations reveal an increase in the main transition temperature by about 25 K relative to GMP, and the occurrence of non-bilayer phases at high temperatures (inverted-cylinder or stacked phases). Structurally, the GDP system tends to evidence a tighter packing of the chains, a reduced extent of tilting, increased order parameters and a reduced fluidity. These differences are easily interpreted in terms of two key changes in molecular properties when going from GMP to GDP: (i) the reduction of the headgroup polarity and hydration (from two free hydroxyl groups to a single one); (ii) the increase in the effective tail cross-section relative to the (hydrated) headgroup cross-section, conferring to GDP a particular wedge shape. These two effects contribute to the relative instability of the liquid-crystalline phase, the stability being recovered in nature when the diglyceride headgroup is functionalized by a bulky or/and polar substituent.

1. Introduction

Lipid bilayers, the main constituent of biological membranes, are of crucial importance to all living organisms because they represent the boundaries defining the different cellular compartments as well as the barrier and first interaction site to the extracellular medium [1].

Aqueous lipid systems can present many different phases [2,3] depending on the types of the lipid molecules, on their concentrations, and on the possible presence of cosolutes, as well as on pressure and temperature. The two biologically most relevant of these phases are bilayer phases, namely the gel (GL) and the liquid crystal (LC) phases [2]. In the GL phase, the aliphatic lipid tails are arranged in nearly all-*trans* conformations and in orientations that are generally tilted with respect to the bilayer normal. In the LC phase, the aliphatic tails are disordered, presenting a mixture of *trans* and *gauche* conformations, and no preferential orientation of the chains (tilting) is observed. Compared to the GL phase, the LC phase is also laterally more expanded and transversely more compact. For a given bilayer composition and under specified environmental conditions, the temperature at which the GL↔LC transition occurs is called the main transition (or melting) temperature T_m. Understanding the influence of composition and environment on this temperature as well as on the properties of the two phases is of fundamental biological and technological importance [2,4].

Atomistic molecular dynamics (MD) simulations have greatly contributed to the characterization and understanding of the structure, thermodynamics and dynamics of lipid bilayers under various conditions [5–26]. These simulations provide information at a spatial (atomic level) and temporal (femtosecond) resolution inaccessible to experiment, concerning system sizes ($\sim$ 10 nm) and timescales ($\sim 1\,\mu$s) already relevant for the evaluation of thermodynamic properties *via* statistical mechanics and the comparison with experimental data. However, the biologically most relevant phospholipids such as dipalmitoylphosphatidylcholine (DPPC) remain relatively challenging to simulate, owing to difficulties in the force-field design [27,28] and treatment of electrostatic interactions

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[27,29–35], and to the slow convergence of system properties with respect to both system size [22,28,36–40] and simulation timescale [36,37,40–42]. For this reason, it is also interesting to consider less complex bilayer systems such as those involving monoglyceride lipids [43–52].

In a series of previous studies by our group, atomistic MD simulations have been used to characterize the phase- and phase-transition properties of a simple saturated monoglyceride, glycerol-1-monopalmitate (GMP; Fig. 1), under various environmental conditions [20–26]. Although biologically less relevant than its DPPC cousin, this model lipid represents an extraordinary testing ground for the exploration of basic bilayer properties and the formulation of qualitative principles governing them.

The next step in complexity from GMP to biologically more relevant lipids such as DPPC is the addition of a second aliphatic tail. Remaining in the context of simple esters of glycerol with palmitic acid (no headgroup functionalization), this corresponds to the two structural isomers of glycerol–dipalmitate (GDP; Fig. 1), namely glycerol-1,3-dipalmitate (13GDP) and glycerol-1,2-dipalmitate (12GDP). Note that 12GDP is a chiral molecule whereas 13GDP is not. Besides representing the simplest model lipids with two tails, diacylglycerides are actually also biologically relevant *per se*. They are involved in the metabolism of lipids [53], modulate the activity of membrane enzymes [54], participate in the transduction of extracellular signals [55], and induce structural changes in biological membranes [54,56]. The properties of diaglycerides have been studied experimentally in the context of both pure crystals and hydrated bilayers [54]. The main transition temperatures T_m for the two GDP isomers are known, namely 72–74°C and 63–66° C for 13GDP and 12GDP, respectively, based on Refs. [56–58]. The T_m values reported in earlier studies may differ by up to 10 K from these estimates [56], but consistently present a T_m difference of about 10 K between the two isomers.

In the present study, GDP bilayer patches consisting of 2×8×8 lipid molecules, either 12GDP (racemic mixture) or 13GDP, are investigated to characterize the effect of two aliphatic chains compared to only one for GMP on the structural, dynamic and thermodynamic properties of the membrane and on its main transition temperature T_m. To this purpose, a set of 67 MD simulations of up to 300 ns duration, carried out at different temperatures in the range of 250–370 K, are reported and compared. The comparison is also performed against two MD simulations of GMP at full hydration carried out at 318 and 338 K, previously reported in Refs. [22,23].

2. Methods

2.1. Molecular dynamics simulations

All MD simulations were performed using the GROMOS MD++ program [59–61], with the 53A6$_{OXY}$ force field [62] for the GMP and GDP molecules, along with the simple point charges (SPC) water model [63]. Detailed simulation and force-field information for GMP can be found in Ref. [23] and its supplementary material, and is easily transferable to GDP. The two simulations of GMP at full hydration considered here for comparison purposes have been previously reported in Ref. [23] (simulations labeled $P_NF_{GL}318$ and $P_NF_{LC}338$ therein) and the simulation details are only provided here for the new GDP simulations.

The simulations were carried out under periodic boundary conditions based on rectangular boxes containing a hydrated GDP bilayer patch of 2×8×8 lipid molecules in the *xy*-plane, leading to a total number of 128 lipid molecules in the systems. In the case of the 12GDP isomer, both leaflets consisted of a racemic mixture of the *R* and *S* enantiomers of the molecule.

Two hydration levels were considered, which are distinguished by the letters F (full) and E (elevated), respectively. The full hydration regime for GMP was defined in previous work [20,22–24] based on the phase diagram of the GMP-water system [20,43], where the main transition temperature T_m is seen to become independent of the hydration level above 6.7 water molecules per lipid. Full hydration means that in the context of a bilayer phase, water molecules added beyond this limit present very weak interactions with the lipids, so that they do not alter anymore the bilayer properties. Since the GDP molecules have two chains, an approximate doubling of the bilayer area was expected and the number of water molecules was also doubled, resulting in n_W = 1706 water molecules. This level of hydration for GDP is also referred to here as full hydration, although in view of the lower polarity of the GDP headgroup compared to that of GMP (one-half *vs.* two hydroxyl groups per tail exposed to the solvent), it is likely to already represent an excess. Some additional simulations with an even more elevated water content were also performed, involving n_W = 2559 water molecules, *i.e.* about 20 water molecules per lipid.

Newton's equations of motion were integrated using the leap-frog scheme [64] with a timestep of 2 fs. All solute bond lengths were constrained by application of the SHAKE procedure [65] with a relative geometric tolerance of 10^{-4}. The full rigidity of the water molecules was enforced by application of the SETTLE procedure [66]. The center of mass translational motion of the computational box was removed every 0.2 ps.

The simulations were carried out in the isothermal-isobaric ensemble with a reference pressure P of 1 bar and reference temperatures T ranging from 250 to 370 K. The temperature was maintained by weakly coupling [67] the solute and solvent degrees of freedom separately to temperature baths at temperature T, using a relaxation time of 0.1 ps. The pressure was maintained by weakly coupling [67] the particle coordinates and box dimensions in the *xy*-plane and along the *z*-axis separately (semi-anisotropic pressure coupling [28]) to a pressure bath at pressure P, using a relaxation time of 0.5 ps and an isothermal compressibility of 4.575×10^{-4} (kJ mol^{-1} nm^{-3})$^{-1}$ as appropriate for biomolecular systems [68].

The non-bonded interactions were calculated using a twin-range scheme [68,69], with short- and long-range cutoff distances set to 0.8 and 1.4 nm, respectively, and an update frequency of five timesteps for the short-range pairlist and intermediate-range interactions. A reaction-field correction [70,71] was applied to account for the mean effect of electrostatic interactions beyond the long-range cutoff distance, using a relative dielectric permittivity of 61 as appropriate for the SPC water model [72]. All simulations were carried out for a duration of at most 300 ns after equilibration, and configurations were saved to file every 10 ps for subsequent analysis.

2.2. Simulated systems

A total number of 67 simulations were carried out, differing by: (*i*) the GDP isomer (13GDP *vs.* 12GDP); (*ii*) the hydration level (F *vs.* E); (*iii*) the initial configuration (GL *vs.* LC *vs.* LC1 – LC3); (*iv*) the temperature T (from 250 to 370 K in steps of 20 K); (*v*) the initial pseudo-random velocities in cases of multiple repeats from a common starting configuration. Only a subset of the possible combinations was considered. The corresponding systems and conditions are summarized in Table 1, which also includes the two previously reported GMP simulations [23] for reference.

All simulations started from the original GL or LC-like structures were carried out at seven different temperatures from 250 to 370 K in steps of 20 K. However, the simulations initiated from alternative LC-like structures LC1 – LC3 (see further below) were only performed at four temperatures between 290 and 350 K, and simulation repeats started from the original GL or LC-like structures